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Data Sheet

NMNAT1 Assay Kit

Catalog # 79642
Size: 96 reactions

Background: Human nicotinamide nucleotide adenylyltransferase 1, also known as NMNAT1, is a member of the nicotinamide-nucleotide adenylyltransferases. NMNAT1 is the most widely expressed of the three genes in the NMNAT family. NMNAT1 catalyzes the formation of NAD (Nicotinamide adenine dinucleotide) from nicotinamide mononucleotide (NMN) and Adenosine Triphosphate (ATP).

DESCRIPTION: The *NMNAT1 Inhibitor Screening Assay Kit* is designed to measure NMNAT1 activity for screening and profiling applications. The NMNAT1 assay kit comes in a convenient 96-well format, with purified recombinant NMNAT1 enzyme, NMNAT1 assay buffer, NMN, and ATP sufficient for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
71090	NMNAT1	1 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
	NMN (10 mM)*	400 µg	-80°C	
	ATP (10 mM)	10 µl	-80°C	
	NMNAT1 Assay Buffer	20 ml	+4°C	
79696	96-well plate, white	1	Room Temp.	

*Resuspend in 110 µl H20 for 10 mM solution

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Kinase-Glo MAX (Promega, #V6071)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

REFERENCES:
Schweiger, M., *et al.* (2001) *FEBS Lett.* **492** (1–2): 95–100.
Emanuelli M., *et al.* (2001) *J Biol Chem.* **276 (1):** 406-12.

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **NMNAT1 assay buffer**, **ATP** and **NMN**.

2) Thaw **NMNAT1** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Calculate the amount of **NMNAT1** required for the assay and dilute enzyme to 0.1 ng/µl with **NMNAT1 assay buffer** (2ng/reaction). Aliquot remaining **NMNAT1** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. ***NMNAT1*** *enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

	Positive Control	Test Inhibitor	Blank
NMNAT1	20 µl	20 µl	–
NMNAT1 assay buffer	–	–	20 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl
NMN and ATP (20 µM) Substrate Mixture	25 µl	25 µl	25 µl
Total	50 µl	50 µl	50 µl

3) Add 20 µl of diluted **NMNAT1** to each well labeled as “Positive Control" and “Test Inhibitor”.

4) To the wells designated as "Blank", add 20 µl of **NMNAT1 assay buffer**.

5) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor”. For the “Positive Control" and “Blank”, add 5 µl of the same solution without inhibitor (Inhibitor buffer). Cover plate with aluminum foil and incubate the plate and incubate at 30˚C for 30 minutes.

6) Resuspend **NMN** in 110 µl H20 for 10 mM solution.

7) Dilute **NMN** and **ATP** in **NMNAT1 assay buffer** to make a 20 µM solution for both **NMN** and **ATP**. For example, add 5 µl **NMN** and 5 µl **ATP** to 2.5 ml **NMNAT1 assay buffer**

8) Initiate reaction by adding 25 µl of substrate mixture to the wells designated "Blank", “Positive Control”, and "Test Inhibitor Control". Cover plate with aluminum foil and incubate the plate and incubate at 30˚C for 30 minutes.

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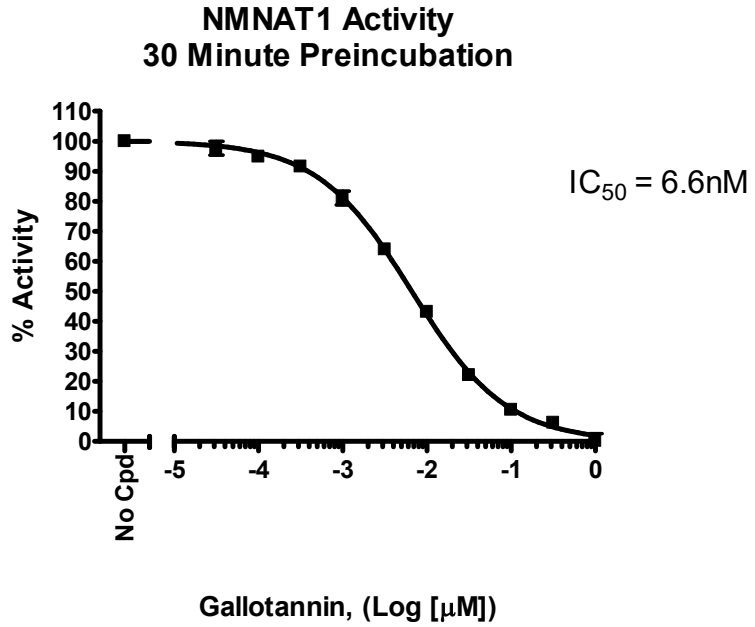
9) Thaw Kinase-Glo Max reagent.

10) After the 30 minute reaction, add 50 µl of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 20 minutes.

11) Measure luminescence using the microplate reader. "Blank" value is subtracted from all readings.

Example of Assay Results:



Inhibition of NMNAT1 enzyme by Gallotannin, measured using the *NMNAT1 assay kit* (Cat. #79642). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
NMNAT1, His-tag	71090	100 µg
NAMPT (PBEF1), GST-tag	91004	50 µg
NAMPT (PBEF1)	71098	50 µg
NAMPT Inhibitor Screening Assay Kit	71276	96 rxns
NAMPT Inhibitor Screening Assay Kit	79307	384 rxns

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